

**AMENDMENT**

**U.S. Appln. No. 09/830,876**

**IN THE CLAIMS:**

**This listing of claims will replace all prior versions and listings of claims in the application:**

**LISTING OF CLAIMS:**

Claim 1. (Currently Amended) A two-site immunoassay for the qualitative or quantitative detection of alpha-amylase in a test sample, said immunoassay comprising;

- (i) exposing said test sample to a first antibody or fragment thereof which specifically or preferentially binds to a first epitope on said alpha-amylase, under conditions permitting binding of said first antibody or fragment thereof to alpha-amylase if present,
- (ii) subsequently exposing bound alpha-amylase, if any, to a second antibody or fragment thereof which specifically or preferentially binds to a second epitope on said alpha-amylase that is distinct from said first epitope, under conditions permitting binding of said second antibody or fragment thereof to said bound alpha-amylase, and
- (iii) detecting any binding of said second antibody or fragment thereof to said bound alpha-amylase,

wherein either of said first or second epitopes is an epitope comprising one or more ~~of the~~ amino acid sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3, and wherein detection of binding of said second antibody or fragment thereof to said bound alpha-amylase

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qualitatively or quantitatively indicates the presence of alpha-amylase in said test sample; ~~IDRLVSIRTRGQIHS (SEQ ID NO: 1), CRDDRPYADG (SEQ ID NO: 2), VNWVNKVGGG (SEQ ID NO: 3) and variants thereof showing  $\geq$  80% sequence identity.~~

Claim 2. (Cancelled).

Claim 3. (Currently Amended) ~~An~~ The immunoassay according to claim 1, wherein either of said first or second epitopes is a conformational epitope comprising one or more of the amino acid sequences; IDRLVSIRTRGQIHS (SEQ ID NO: 1), CRDDRPYADG (SEQ ID NO: 2), VNWVNKVGGG (SEQ ID NO: 3).

Claim 4. (Currently Amended) ~~An~~ The immunoassay according to claim 1, wherein either of said first or second epitopes is a conformational epitope comprising all of the amino acid sequences; IDRLVSIRTRGQIHS (SEQ ID NO: 1), CRDDRPYADG (SEQ ID NO: 2), VNWVNKVGGG (SEQ ID NO: 3).

Claim 5. (Currently Amended) ~~An~~ The immunoassay according to claim 1, wherein said first antibody or fragment thereof or said second antibody or fragment thereof is ~~provided~~ bound to a solid support.

Claim 6. (Currently Amended) ~~An~~ The immunoassay according to claim 5, wherein the solid support is selected from microwell plates, membranes, beads, particles, sensors and porous test strips.

Claim 7. (Currently Amended) ~~An~~ The immunoassay according to claim 1, wherein binding of the second antibody or fragment thereof to alpha-amylase is detected through the use of a readily detectable label.

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Claim 8. (Currently Amended) ~~An~~ The immunoassay according to claim 7, wherein the detectable label is selected from detectable enzymes, radioisotopes, luminescent labels and fluorescent labels.

Claim 9. (Currently Amended) ~~An~~ The immunoassay according to claim 1, wherein binding of the second antibody or fragment thereof to alpha-amylase is detected through the use of immunochromatography or agglutination.

Claim 10. (Currently Amended) ~~An~~ The immunoassay according to claim 1, wherein at least one of the first and second antibodies or fragments thereof is ~~selected from a monoclonal antibody antibodies—or fragments—fragment~~ thereof—and ~~recombinant antibody fragments~~.

Claim 11. (Currently Amended) ~~An~~ The immunoassay according to claim 1, wherein the test sample is obtained from a cereal grain.

Claim 12. (Currently Amended) ~~An~~ The immunoassay according to claim 11, wherein the cereal grain is selected from the group consisting of bread wheat (*Triticum aestivum*), durum wheat (*Triticum turgidum* var. durum), club wheat (*Triticum compactus*), rye (*Secale cereale*), triticale (*Triticosecale* species) and barley (*Hordeum vulgare*).

Claim 13. (Currently Amended) ~~An~~ The immunoassay according to claim 11, wherein the test sample is an aqueous extract from grain, grain meal or flour.

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Claim 14. (Currently Amended) ~~An~~ The immunoassay according to claim 1, wherein said immunoassay provides for the quantitative detection of alpha-amylase by further comprising:

(iv) comparing the level of detected binding of the second antibody or fragment thereof ~~to alpha-amylase against a suitable standard~~ for the test sample to the levels of detected binding of said second antibody or fragment thereof to samples having known alpha-amylase enzyme activities, or Falling Numbers thereby providing for quantitative detection of alpha-amylase in said test sample.

Claims 15-22. (Cancelled).

Claim 23. (New) The immunoassay of claim 1, wherein the test sample comprises grain meal or flour.

Claim 24. (New) The method of claim 13, wherein the aqueous extract comprises NaCl or CaCl<sub>2</sub>.

Claim 25. (New) A process for determining weather damage in a plant or crop said process comprising performing the method of claim 1 on one or more test samples selected from the group consisting of grain, grain meal, flour, an aqueous extract of grain, an aqueous extract of grain meal and an aqueous extract of flour, wherein said test sample is obtained from said plant or crop and wherein the presence of alpha-amylase in said test sample as determined by the level of detected binding of the second antibody or fragment thereof to the test sample indicates that the plant or crop has been weather damaged.

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Claim 26. (New) The process of claim 25, wherein the test sample is obtained from a cereal grain.

Claim 27. (New) The process of claim 26, wherein the cereal grain is selected from the group consisting of bread wheat (*Triticum aestivum*), durum wheat (*Triticum turgidum* var. durum), club wheat (*Triticum compactus*), rye (*Secale cereale*), triticales (*Triticosecale* species) and barley (*Hordeum vulgare*).

Claim 28. (New) The process of claim 25, wherein the test sample is an aqueous extract from grain, grain meal or flour.

Claim 29. (New) The process of claim 25, further comprising quantifying the amount of alpha-amylase in the test by a process comprising comparing the level of detected binding of the second antibody or fragment thereof for the test sample to the levels of detected binding of said second antibody or fragment thereof to samples having known alpha-amylase enzyme activities, viscosities, or Falling Numbers thereby quantifying the amount of alpha-amylase in said test sample.

Claim 30. (New) The immunoassay of claim 1, wherein said immunoassay provides for the qualitative detection of alpha-amylase by further comprising:

- (iv) comparing the level of detected binding of the second antibody or fragment thereof for the test sample to the levels of detected binding of said second antibody or fragment thereof to samples having known viscosities thereby providing for quantitative detection of alpha-amylase in said test sample.